Perkins 1975

JOB PROGRESS REPORT

STATE: California

COOPERATORS: U.S. Fish and Wildlife Service

PROJECT NO.: W-52-R-19 PROJECT TITLE: W/L Investigations Laboratory

JOB NO.: I-3 JOB TITLE: Study of Waterfowl Botulism

PERIOD COVERED: July 1, 1974 to June 30, 1975

SUMMARY:

A total of 55 invertebrate samples were collected from July 1, 1974 to June 30, 1975. Two samples were toxic out of five analyzed.

The four year contract for botulism research ended June 30, 1974 so collection of invertebrates on the Lower Klamath National Wildlife Refuge was limited to two trappings. The trapping resulted in collection of 50 samples kept for analysis.

Approximately 25,000 birds died from botulism in California from July 1974-June 1975.

BACKGROUND:

Waterfowl botulism is a form of food poisoning which during the past eight years has been directly responsible for the death of over 325,000 waterfowl and shorebirds in California. With an increased understanding of the disease, management techniques have been improved significantly. However, while management practices are essential, the ultimate realistic goal in controlling a disease is virtual prevention. By increasing our knowledge of the causative organism, outbreak control may be improved.

The cost of past outbreaks has been high both in terms of the resource and dollars. Because of this intolerable cost, research efforts were directed at the cause and management of botulism outbreaks.

OBJECTIVES:

The purpose of this research project was to gain a better understanding of the life cycle of Clostridium botulinum type C as it pertains to waterfowl mortalities. Since current research has indicated invertebrates may play a significant role in botulism outbreaks invertebrates were collected from the Lower Klamath NWR and kept for testing for the presence of spores and toxin. Improved management and mortality control techniques were the ultimate goals of the project.

PROCEDURES:

Polypropylene light traps were used for collecting free swimming aquatic invertebrates. Collections consisted almost entirely of invertebrates which were easily sorted into bioassay samples. Using this trap we caught immature and adult insects of the orders Hemiptera (Corixidae, Notonectidae), Coleoptera (Hydrophilidae, Dytiscidae), Odonata (immature only), stages of the crustacean order Cladocera, Diptera (Chironomidae), Hydrocarina (water mites) and Cyprinidae (Tui chub).

Laboratory analysis consisted of sample identification to the family level and qualitative toxicity testing.

The procedures for testing samples for the presence of type C toxin or cells were as follows:

1. Toxicity testing without incubation.

   a) 1.0 g of sample was weighed.

   b) The sample was placed in a mortar and distilled water was added – the volume of water was either equal to or a multiple of the weight, 2.0 ml was usually satisfactory.

   c) The sample was well ground (mortar and pestle).

   d) Sample allowed to set for about 30 minutes.

   e) All material was poured from the mortar into a tube and centrifuged at 27,000 x g for ten minutes at 4°C.

   f) 1.0 ml of supernate from the centrifuged sample was added to 9.0 ml of sterile distilled water.

   g) The 10 ml of sample was shaken and then filter* sterilized (0.45 μ).

   h) The mouse protection test was used for all toxin detection. Two mice were used for each test, each received 1.0 ml of sterile filtrate intraperitoneally (I.P.) and one of the mice also received 0.5 ml of C. botulinum type C antitoxin (I.P.).

2. Toxicity testing following incubation in Brewer's thioglycollate.

   a) Tubes of Brewer's thioglycollate** were heated before sample inoculation.

   b) The invertebrate bodies were punctured prior to addition to the media.

   c) Samples were incubated for six days at 32°C.

   d) After incubation 10 ml of fluid was removed from each tube and centrifuged at 27,000 x g for ten minutes at 4°C.

   e) The supernate was filter sterilized (0.45 μ) and tested for toxin.

When only 1.0 g of sample was available procedure #1 was used. With samples of less than 1.0 g procedure #2 was used and when more than 1.0 g was available both techniques were used.

---

*Nalgene filter units, Sybron Corporation, Rochester, New York.
**BBL Division of BioQuest, P.O. Box 175, Cockeysville, Maryland.

Laboratory personnel inspected each area where major botulism losses occurred. Airboats, four-wheel drive vehicles, labrador retrievers and walking were used to aid in estimating actual waterfowl losses and in collecting diagnostic and research samples.

FINDINGS:

A total of 55 samples were collected. These included 50 from the Lower Klamath NWR, 3 from Lake Almanor, one from Merced Sewer Farm and one from South San Francisco Bay.

To date, 2 samples have been found toxic, both were Diptera larvae (maggots).

Table 1 shows the sample, date, location, specific composition, volume, treatment and results of toxicity testing.

Use of light traps for the collection of aquatic invertebrates from the Lower Klamath NWR resulted in the trapping of immature and adult individuals of the orders: Hemiptera (families Corixidae and Notonectidae), Coleoptera (families Hydrophilidae and Dytiscidae), Odonata (immature only), Hydrocarina, stages of the crustacean order Cladocera, Diptera (Chironomidae) and Cyprinidae (tui chub).

The botulism losses actually picked up during the period July 1, 1974-June 30, 1975 were:

Area	Birds Dead
Tule Lake	26
Lower Klamath NWR	965
Lake Almanor	532
Sacramento Refuge System	560
San Francisco Bay	13,000
Stockton Sewer Ponds	5,385
Kern NWR, Pixley NWR & Tulare Lake Basin	135
Salton Sea NWR	855
Miscellaneous	551
Total	22,009

In addition to the dead birds, there were sick birds picked up and treated. Approximately 240 shorebirds and ducks were treated at the duck hospital located on the Salton Sea NWR, about 50 birds were treated on the Kern NWR and near Buttonwillow.

The number of birds actually dying from botulism would be higher than 22,000; an estimated 25,000 would represent the total.

RECOMMENDATIONS:

An effort has been made over the past four years to determine the invertebrates most likely to be the source of botulism toxin to waterfowl. Even though large numbers of invertebrates have been tested maggots are most consistently toxic. We think maggots were responsible for the south bay outbreak. Invertebrates need to be collected during a large outbreak to determine distribution and availability of toxin and/or spores to waterfowl.

Further research is needed to more clearly define the ecological requirements of toxin production. We still do not have a complete understanding of this phase, which is a prerequisite to further improving field control methods.

The orientation of research for this project has been to determine which invertebrates can support botulism toxin production and to determine the significance of various natural toxin sources to the waterfowl. Our approach will therefore remain the same.

Prepared by: Patricia Perkins
Patricia Perkins
Laboratory Tech. II

Approved by: Brian Hunter
B. F. Hunter
Acting Project Leader

Approved by: E. G. Hunt
E.G. Hunt, Chief
W/L Management Branch

Date: AUG 7 1975